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L5: Entry 2 of 14 File: PGPB Jul 5, 2001

PGPUB-DOCUMENT-NUMBER: 20010006639
PGPUB-FILING-TYPE: new-utility
DOCUMENT-IDENTIFIER: US 20010006639 A1

TITLE: Immunogenic peptides from the HPV E7 protein

PUBLICATION-DATE: July 5, 2001

US-CL-CURRENT: 424/186.1; 424/450, 435/320.1, 530/326, 536/23.72

APPL-NO: 09/ 759960 [PALM]
DATE FILED: January 12, 2001

RELATED-US-APPL-DATA:

RLAN	RLFD	RLPC	RLKC	RLAC
09759960	Jan 12, 2001	GRANTED	A1	US
09169425	Oct 9, 1998			US
6183746	Oct 9, 1997			US
60061657				

[0001] This application claims priority from currently pending U.S. Ser. No. 60/061,657 (herein incorporated by reference), which was filed Oct. 9, 1997.

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L5: Entry 2 of 14 File: PGPB Jul 5, 2001

PGPUB-DOCUMENT-NUMBER: 20010006639
PGPUB-FILING-TYPE: new-utility
DOCUMENT-IDENTIFIER: US 20010006639 A1

TITLE: Immunogenic peptides from the HPV E7 protein

PUBLICATION-DATE: July 5, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Chiez, Roman M.	Belmont	MA	US	
Collins, Edward J.	Carrboro	NC	US	
Hedley, Mary Lynne	Lexington	MA	US	

US-CL-CURRENT: 424/186.1; 424/450, 435/320.1, 530/326, 536/23.72

CLAIMS:

What is claimed is:

1. A peptide less than 19 amino acids in length, wherein the peptide comprises the amino sequence Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:16).

2. The peptide of claim 1, wherein the peptide's amino acid sequence comprises Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:3).

3. The peptide of claim 1, wherein the peptide's sequence comprises Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

4. The peptide of claim 3, wherein Xaa is Ala or Met.

5. The peptide of claim 1, wherein the peptide's sequence comprises Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys Ser Gln Lys (SEQ ID NO:25).

6. A peptide less than 19 amino acids in length, wherein the peptide comprises the amino acid sequence Gly Thr Leu Gly Ile Val Cys Pro Ile (SEQ ID NO:21).

7. The peptide of claim 6, wherein the peptide's sequence comprises Xaa Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:25).

8. The peptide of claim 6, wherein the peptide's sequence comprises Met Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:26).

9. The peptide of claim 7, wherein the peptide's sequence consists of Xaa Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu.

10. The peptide of claim 8, wherein the peptide's sequence consists of Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys Ser Gln Lys (SEQ ID NO: 26).

11. A peptide consisting of the amino acid sequence Thr Leu Gly Ile Val Cys Pro Ile (SEQ ID NO:20).

12. A polypeptide comprising a first peptide and a second peptide linked by a peptide bond, the first peptide being a peptide which controls intracellular trafficking of a peptide to which it is attached, and the second peptide consisting of a sequence 12-18 amino acids in length comprising the sequence Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:16).

13. The polypeptide of claim 12, wherein the sequence of the first peptide comprises the amino acid sequence Met Ala Ile Ser Gly Val Pro Val Leu Gly Phe Phe Ile Ile Ala Val Leu Met Ser Ala Gln Glu Ser Trp Ala (SEQ ID NO:18).

14. The polypeptide of claim 12, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

15. The polypeptide of claim 12, wherein the amino acid sequence of the second polypeptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

16. The polypeptide of claim 13, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

17. The polypeptide of claim 13, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

18. A polypeptide comprising a first peptide and a second peptide linked by a peptide bond, the first peptide being a peptide which controls intracellular trafficking of a peptide to which it is attached, and the second peptide consisting of a sequence 8-18 amino acids in length comprising the sequence Thr Leu Gly Ile Val Cys Pro Ile (SEQ ID NO:20).

19. A therapeutic composition comprising (a) the peptide of claim 1, and (b) a pharmaceutically acceptable carrier.

20. A therapeutic composition comprising (a) the peptide of claim 6, and (b) a pharmaceutically acceptable carrier.

21. A microparticle comprising a polymeric matrix and the peptide of claim 1.

22. A microparticle comprising a polymeric matrix and the peptide of claim 6.

23. A microparticle comprising a polymeric matrix and the polypeptide of claim 1.

24. A microparticle comprising a polymeric matrix and the polypeptide of claim 18.

25. A liposome or immune-stimulating complex (ISCOM) containing the peptide of claim 1.

26. A liposome or immune-stimulating complex (ISCOM) containing the peptide of claim 6.

27. A method of eliciting an MHC class I-mediated immune response in a mammal, which method comprises administering a purified preparation of the peptide of claim 1 to a mammal.

28. A method of eliciting an MHC class I-mediated immune response in a mammal, which method comprises administering a purified preparation of the peptide of claim 6 to a mammal.

29. A method of eliciting an MHC class I-mediated immune response in a mammal, which method comprises administering the microparticle of claim 21 to a mammal.

30. The method of claim 29, wherein the polymeric matrix of said microparticle consists essentially of a copolymer of poly-lactic-co-glycolic acid (PLGA).

31. A method of eliciting an MHC class I-mediated immune response in a mammal, which method comprises administering the microparticle of claim 22 to a mammal.

32. The method of claim 31, wherein the polymeric matrix of said microparticle consists essentially of a copolymer of poly-lactic-co-glycolic acid (PLGA).

33. A nucleic acid comprising a coding sequence coding for expression of the peptide of claim 1.

34. A nucleic acid comprising a coding sequence coding for expression of the peptide of claim 6.

35. A nucleic acid comprising a coding sequence coding for expression of the polypeptide of claim 12.

36. A nucleic acid comprising a coding sequence coding for expression of the polypeptide of claim 18.

37. A plasmid comprising a coding sequence coding for expression of the polypeptide of claim 12.

38. A microparticle comprising a polymeric matrix and the plasmid of claim 37.

39. The microparticle of claim 38, wherein the polymeric matrix of the microparticle consists essentially of a copolymer of PLGA.

40. The microparticle of claim 38, wherein the microparticle has a diameter of 0.02 to 20 microns.

41. The microparticle of claim 38, wherein the microparticle has a diameter of less than about 11 microns.

42. A cell comprising the plasmid of claim 37.

43. The cell of claim 42, wherein the cell is a mammalian B cell or APC.

44. A method of making a polypeptide, which method comprises maintaining the cell of claim 42 under conditions permitting expression of said polypeptide.

45. A plasmid comprising a coding sequence coding for expression of the polypeptide of claim 18.

46. A microparticle comprising a polymeric matrix and the plasmid of claim 45.

47. The microparticle of claim 46, wherein the polymeric matrix of said microparticle consists essentially of a copolymer of PLGA.

48. The microparticle of claim 46, wherein the microparticle has a diameter of 0.02 to 20 microns.

49. The microparticle of claim 46, wherein the microparticle has a diameter of less than about 11 microns.

50. A cell comprising the plasmid of claim 45.

51. The cell of claim 50, wherein the cell is a mammalian B cell or APC.

52. A method of making a peptide, which method comprises maintaining the cell of claim 50 under conditions permitting expression of said polypeptide.

53. A method of inducing an immune response in a mammal, which method comprises administering the nucleic acid of claim 35 to a mammal.

54. A method of inducing an immune response in a mammal, which method comprises administering the nucleic acid of claim 36 to a mammal.

55. A method of inducing an immune response in a mammal, which method comprises administering the plasmid of claim 37 to a mammal.

56. A method of inducing an immune response in a mammal, which method comprises administering the plasmid of claim 45 to a mammal.

57. A method of inducing an immune response in a mammal, which method comprises administering the microparticle of claim 38 to a mammal.

58. The method of claim 57, wherein the mammal is a human.

59. The method of claim 58, wherein the human suffers from, or is at risk of a condition selected from the group consisting of exophytic condyloma, flat condyloma, cervical cancer, respiratory papilloma, conjunctival papilloma, genital-tract HPV infection, and cervical dysplasia.

60. A method of inducing an immune response in a mammal, which method comprises administering the microparticle of claim 46 to a mammal.

61. The method of claim 60, wherein the mammal is a human.

62. The method of claim 61, wherein the human suffers from, or is at risk of, a condition selected from the group consisting of exophytic condyloma, flat condyloma, cervical cancer, respiratory papilloma, conjunctival papilloma, genital-tract HPV infection, and cervical dysplasia.

63. A plasmid DNA comprising the sequence of SEQ ID NO:7.

64. A microparticle comprising a polymeric matrix and a nucleic acid, wherein the polymeric matrix consists essentially of PLGA and the nucleic acid comprises the sequence of SEQ ID NO:7.

65. A method of inducing a cell mediated, anti-HPV immune response in a mammal, which method comprises administering to the mammal a DNA comprising the sequence of SEQ ID NO:7.

66. A method of inducing an immune response in a patient, which method comprises administering to the patient a microparticle having a diameter of less than 20 microns and consisting essentially of a polymeric matrix and a nucleic acid molecule, wherein the polymeric matrix consists essentially of PLGA and the nucleic acid molecule comprises the sequence of SEQ ID NO:7.

67. A DNA comprising the sequence of SEQ ID NO:5.

68. A DNA comprising the sequence of nucleotides 3219-3624 of SEQ ID NO:7.

69. A DNA comprising the sequence of nucleotides 3290-3413 of SEQ ID NO:7.

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L5: Entry 4 of 14 File: USPT May 22, 2001

US-PAT-NO: 6235523
DOCUMENT-IDENTIFIER: US 6235523 B1

TITLE: Vectors for DNA immunization against cervical cancer

DATE-ISSUED: May 22, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Cao; Shi-Xian	Etobicoke			CAX
Klein; Michel H.	Willowdale			CAX
Tartaglia; James	Schenectady	NY		
Moingeon; Phillipe	F-Pommiers			FRX
Rovinski; Benjamin	Thornhill			CAX

US-CL-CURRENT: 435/320.1; 424/186.1, 424/192.1, 424/204.1, 514/44, 536/23.72

CLAIMS:

What we claim is:

1. A vector comprising a nucleic acid molecule encoding an HPV-16 E7 antigen lacking amino acids 21-26, wherein said nucleic acid molecule is operatively linked to a cytomegalovirus promoter.

2. The vector of claim 1 wherein said nucleic acid molecule is inserted into plasmid CMV-3.

3. The vector of claim 1 wherein said vector is pCMV-dE7.

4. A vector comprising a nucleic acid molecule encoding HPV-16 E7 antigen epitopes consisting of amino acids 11 to 20, 49 to 57, 82 to 90 and 86 to 93 and an HPV-16 E6 antigen epitope consisting of amino acids 29 to 38, wherein said nucleic acid molecule is operatively linked to a cytomegalovirus promoter.

5. The vector of claim 4 wherein said nucleic acid molecule consists of SEQ ID No: 4 or 5.

6. The vector of claim 4 wherein said nucleic acid molecule encodes an amino acid sequence consisting of SEQ ID No: 6.

7. The vector of claim 4 wherein said vector is pCMV3-HPVT#1.

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Patent Number Information

Application Number: **08/787547** **Order This File** **Assignments**
Examiner Number: **72792 / BRUSCA, JOHN**
Filing Date: **01/22/1997**
Group Art Unit: **1636**
Application Received: **01/22/1997**
Class/Subclass: **514/044.000**
Patent Number: **5783567**
Lost Case: **NO**
Issue Date: **07/21/1998**
Interference Number:
Date of Abandonment: **00/00/0000**
Unmatched Petition:
Attorney Docket Number: **08191/003001**
L&R Code:
Status: **150 / PATENTED CASE**
Status Date: **06/15/1998**
Confirmation Number: **6646**
Title of Invention: **MICROPARTICLES FOR DELIVERY OF NUCLEIC ACID**

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08787547	9200 FILE REPOSITORY (FRANCONIA)	02/11/2002		No Charge to Name	ABAIG	

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L5: Entry 6 of 14 File: USPT Feb 6, 2001

US-PAT-NO: 6183746
DOCUMENT-IDENTIFIER: US 6183746 B1

TITLE: Immunogenic peptides from the HPV E7 protein

DATE-ISSUED: February 6, 2001

INVENTOR-INFORMATION:

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Collins; Edward J.	Carrboro	NC		
Hedley; Mary Lynne	Lexington	MA		

US-CL-CURRENT: 424/186.1; 424/185.1, 424/199.1, 424/204.1, 424/450, 424/489, 435/235.1, 435/320.1, 435/326, 435/829, 514/44, 536/23.72

CLAIMS:

What is claimed is:

1. A method of making a polypeptide, which method comprises maintaining a cell containing a plasmid comprising a coding sequence coding for expression of a polypeptide under conditions permitting expression of said polypeptide, the polypeptide comprising a first peptide and a second peptide linked by a peptide bond, the first peptide being a peptide which controls intracellular trafficking of a peptide to which it is attached, and the second peptide consisting of a sequence 12-18 amino acids in length comprising the sequence Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:16).

2. A method of making a polypeptide, which method comprises maintaining a cell containing a plasmid comprising a coding sequence coding for expression of a polypeptide under conditions permitting expression of said polypeptide, the polypeptide comprising a first peptide and a second peptide linked by a peptide bond, the first peptide being a peptide which controls intracellular trafficking of a peptide to which it is attached, and the second peptide consisting of a sequence 8-18 amino acids in length comprising the sequence Thr Leu Gly Ile Val Cys Pro Ile (SEQ ID NO:20).

3. A method of inducing an immune response in a mammal, which method comprises administering to a mammal a nucleic acid comprising a coding sequence coding for expression of a polypeptide comprising a first peptide and a second peptide linked by a peptide bond, the first peptide being a peptide which controls intracellular trafficking of a peptide to which it is attached, and the second peptide consisting of a sequence 12-18 amino acids in length comprising the sequence Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:20).

4. A method of inducing an immune response in a mammal, which method comprises administering to a mammal a nucleic acid comprising a coding sequence coding for expression of a polypeptide comprising a first peptide and a second peptide linked by a peptide bond, the first peptide being a peptide which controls intracellular trafficking of a peptide to which it is attached, and the second peptide consisting

of a sequence 8-18 amino acids in length comprising the sequence Thr Leu Gly Ile Val Cys Pro Ile (SEQ ID NO:20).

5. A method of inducing an immune response in a mammal, which method comprises administering to a mammal a plasmid comprising a coding sequence coding for expression of a polypeptide comprising a first peptide and a second peptide linked by a peptide bond, the first peptide being a peptide which controls intracellular trafficking of a peptide to which it is attached, and the second peptide consisting of a sequence, 12-18 amino acids in length comprising the sequence Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:16).

6. A method of inducing an immune response in a mammal, which method comprises administering to a mammal a plasmid comprising a coding sequence coding for expression of a polypeptide comprising a first peptide and a second peptide linked by a peptide bond, the first peptide being a peptide which controls intracellular trafficking of a peptide to which it is attached, and the second peptide consisting of a sequence 8-18 amino acids in length comprising the sequence Thr Leu Gly Ile Val Cys Pro Ile (SEQ ID NO:20).

7. A method of inducing an immune response in a mammal, which method comprises administering to a mammal a microparticle comprising

(a) a polymeric matrix; and

(b) a plasmid comprising a coding sequence coding for expression of a polypeptide comprising a first peptide and a second peptide linked by a peptide bond, the first peptide being a peptide which controls intracellular trafficking of a peptide to which it is attached, and the second peptide consisting of a sequence 12-18 amino acids in length comprising the sequence Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:16).

8. The method of claim 7, wherein the mammal is a human.

9. The method of claim 8, wherein the human suffers from, or is at risk of a condition selected from the group consisting of exophytic condyloma, flat condyloma, cervical cancer, respiratory papilloma, conjunctival papilloma, genital-tract HPV infection, and cervical dysplasia.

10. A method of inducing an immune response in a mammal, which method comprises administering to a mammal a microparticle comprising

(a) a polymeric matrix; and

(b) a plasmid comprising a coding sequence coding for expression of a polypeptide comprising a first peptide and a second peptide linked by a particle bond, the first peptide being a peptide which controls intracellular trafficking of a peptide to which it is attached, and the second peptide consisting of a sequence 8-18 amino acids in length comprising the sequence Thr Leu Gly Ile Val Cys Pro Ile (SEQ ID NO:20).

11. The method of claim 10, wherein the mammal is a human.

12. The method of claim 11, wherein the human suffers from, or is at risk of, a condition selected from the group consisting of exophytic condyloma, flat condyloma, cervical cancer, respiratory papilloma, conjunctival papilloma, genital-tract HPV infection, and cervical dysplasia.

13. A method of inducing a cell mediated, anti-HPV immune response in a mammal, which method comprises administering to the mammal a DNA comprising the sequence of SEQ ID NO:7.

14. A method of inducing an immune response in a patient, which method comprises administering to the patient a microparticle having a diameter of less than 20 microns and consisting essentially of a polymeric matrix and a nucleic acid molecule, wherein the polymeric matrix consists essentially of PLGA and the nucleic

acid molecule comprises the sequence of SEQ ID NO:7.

15. A method of inducing an immune response in an mammal, which method comprises administering a nucleic acid comprising a coding sequence coding for expression of a peptide less than 19 amino acids in length, wherein the peptide comprises the amino acid sequence Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:16).

16. The method of claim 15, wherein the peptide's amino acid sequence comprises Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:3).

17. The method of claim 15, wherein the peptide's amino acid sequence comprises Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

18. The method of claim 17, wherein Xaa is Ala or Met.

19. The method of claim 15, wherein the peptide's amino acid sequence comprises Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys Ser Gln Lys (SEQ ID NO:25).

20. A method of inducing an immune response in an mammal, which method comprises administering a nucleic acid comprising a coding sequence coding for expression of a peptide less than 19 amino acids in length, wherein the peptide comprises the amino acid sequence Gly Thr Leu Gly Ile Val Cys Pro Ile (SEQ ID NO:21).

21. The method of claim 20, wherein the peptide's amino acid sequence comprises Xaa Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:31).

22. The method of claim 20, wherein the peptide's amino acid sequence comprises Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:33).

23. The method of claim 21, wherein the peptide's amino acid sequence consists of Xaa Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:31).

24. The method of claim 22, wherein the peptide's amino acid sequence consists of Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys Ser Gln Lys (SEQ ID NO:32).

25. The method of claim 1, wherein the sequence of the first peptide comprises the amino acid sequence Met Ala Ile Ser Gly Val Pro Val Leu Gly Phe Phe Ile Ile Ala Val Leu Met Ser Ala Gln Glu Ser Trp Ala (SEQ ID NO: 18).

26. The method of claim 1, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

27. The method of claim 1, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

28. The method of claim 25, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

29. The method of claim 25, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

30. The method of claim 3, wherein the sequence of the rirst peptide comprises the amino acid sequence Met Ala Ile Ser Gly Val Pro Val Leu Gly Phe Phe Ile Ile Ala Val Leu Met Ser Ala Gln Glu Ser Trp Ala (SEQ ID NO: 18).

31. The method of claim 3, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

32. The method of claim 3, wherein the amino acid sequence of the second peptide is

Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

33. The method of claim 30, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

34. The method of claim 30, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

35. The method of claim 5, wherein the sequence of the first peptide comprises the amino acid sequence Met Ala Ile Ser Gly Val Pro Val Leu Gly Phe Phe Ile Ile Ala Val Leu Met Ser Ala Gln Glu Ser Trp Ala (SEQ ID NO: 18).

36. The method of claim 5, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

37. The method of claim 5, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

38. The method of claim 35, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

39. The method of claim 35, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

40. The method of claim 7, wherein the polymeric matrix consists essentially of PLGA.

41. The method of claim 7, wherein rhe microparticle has a diameter of 0.02 to 20 microns.

42. The method of claim 7, wherein the microparticle has a diameter of less than about 11 microns.

43. The method of claim 7, wherein the sequence of the first peptide comprises the amino acid sequence Met Ala Ile Ser Gly Val Pro Val Leu Gly Phe Phe Ile Ile Ala Val Leu Met Ser Ala Gln Glu Ser Trp Ala (SEQ ID NO: 18).

44. The method of claim 7, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

45. The method of claim 7, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID No:4).

46. The method of claim 43, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

47. The method of claim 43, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

48. The method of claim 40, wherein the sequence of the first peptide comprises the amino acid sequence Met Ala Ile Ser Gly Val Pro Val Leu Gly Phe Phe Ile Ile Ala Val Leu Met Ser Ala Gln Glu Ser Trp Ala (SEQ ID NO: 18).

49. The method of claim 40, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

50. The method of claim 40, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

51. The method of claim 48, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

52. The method of claim 48, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

53. The method of claim 42, wherein the sequence of the first peptide comprises the amino acid sequence Met Ala Ile Ser Gly Val Pro Val Leu Gly Phe Phe Ile Ile Ala Val Leu Met Ser Ala Gln Glu Ser Trp Ala (SEQ ID NO: 18).

54. The method of claim 42, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, xaa being Met, Leu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

55. The method of claim 42, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

56. The method of claim 53, wherein the amino acid sequence of the second peptide is Xaa Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys, Xaa being Met, Letu, Ala, Ser, Arg, Lys, Gly, Gln, Asp, or Glu (SEQ ID NO:19).

57. The method of claim 53, wherein the amino acid sequence of the second peptide is Ala Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys (SEQ ID NO:4).

58. The method of claim 10, wherein the polymeric matrix consists essentially of PLGA.

59. The method of claim 10, wherein the microparticle has a diameter of 0.02 to 20 microns.

60. The method of claim 10, wherein the microparticle has a diameter of less than about 11 microns.

61. The method of claim 1, wherein the cell is a mammalian B cell or APC.

62. The method of claim 25, wherein the cell is a mammalian B cell or APC.

63. The method of claim 26, wherein the cell is a mammalian B cell or APC.

64. The method of claim 27, wherein the cell is a mammalian B cell or APC.

65. The method of claim 3, wherein the mammal is a human.

66. The method of claim 4, wherein the mammal is a human.

67. The method of claim 5, wherein the mammal is a human.

68. The method of claim 6, wherein the mammal is a human.

69. The method of claim 65, wherein the human suffers from, or is at risk of a condition selected from the group consisting of exophytic condyloma, flat condyloma, cervical cancer, respiratory papilloma, conjunctival papilloma, genital-tract HPV infection, and cervical dysplasia.

70. The method of claim 66, wherein the human suffers from, or is at risk of a condition selected from the group consisting of exophytic condyloma, flat condyloma, cervical cancer, respiratory papilloma, conjunctival papilloma, genital-tract HPV infection, and cervical dysplasia.

71. The method of claim 67, wherein the human suffers from, or is at risk of a condition selected from the group consisting of exophytic condyloma, flat condyloma, cervical cancer, respiratory papilloma, conjunctival papilloma, genital-tract HPV infection, and cervical.

72. The method of claim 68, wherein the human suffers from, or is at risk of a condition selected from the group consisting of exophytic condyloma, flat condyloma, cervical cancer, respiratory papilloma, conjunctival papilloma, genital-tract HPV infection, and cervical dysplasia.

73. A method of inducing a cell-mediated, anti-HPV immune response in a mammal, which method comprises administering to the mammal a DNA comprising the sequence of SEQ ID NO:5.

74. A method of inducing an immune response in a patient, which method comprises administering to the patient a microparticle having a diameter of less than 20 microns and consisting essentially of a polymeric matrix and a nucleic acid molecule, wherein the polymeric matrix consists essentially of PLGA and the nucleic acid molecule comprises the sequence of SEQ ID NO:5.

75. A method of inducing a cell-mediated, anti-HPV immune response in a mammal, which method comprises administering to the mammal a DNA comprising the sequence of nucleotides 3290-3413 of SEQ ID NO:7.

76. A method of inducing an immune response in a patient, which method comprises administering to the patient a microparticle having a diameter of less than 20 microns and consisting essentially of a polymeric matrix and a nucleic acid molecule, wherein the polymeric matrix consists essentially of PLGA and the nucleic acid molecule comprises the sequence nucleotides 3290-3413 of SEQ ID NO:7.

77. The method of claim 75, wherein the DNA comprises the sequence of nucleotides 3219-3624 of SEQ ID NO:7.

78. The method of claim 77, wherein the nucleic acid molecule comprises the sequence of nucleotides 3219-3624 of SEQ ID NO:7.

79. The method claim 73, wherein the mammal is a human.

80. The method claim 75, wherein the mammal is a human.

81. The method claim 77, wherein the mammal is a human.

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L5: Entry 13 of 14 File: USPT Jul 21, 1998

DOCUMENT-IDENTIFIER: US 5783567 A
TITLE: Microparticles for delivery of nucleic acid

Abstract Paragraph Left (1):
Disclosed is a preparation of microparticles made up of a polymeric matrix and a nucleic acid expression vector. The polymeric matrix includes one or more synthetic polymers having a solubility in water of less than about 1 mg/l. At least 90% of the microparticles have a diameter less than about 100 microns. The nucleic acid is either RNA, at least 50% of which is in the form of closed circles, or circular DNA plasmid molecules, at least 50% of which are supercoiled.

Brief Summary Paragraph Right (5):
The invention is based on the discovery that microparticles containing nucleic acids having an appropriate size for phagocytosis can be made without adversely affecting nucleic acid integrity. These microparticles are highly effective vehicles for the delivery of polynucleotides into phagocytic cells.

Brief Summary Paragraph Right (6):
In general, the invention features a preparation of microparticles, each of which includes a polymeric matrix and a nucleic acid expression vector. The polymeric matrix includes one or more synthetic polymers having a solubility in water of less than about 1 mg/l; in the present context, synthetic is defined as non-naturally occurring. At least 90% of the microparticles have a diameter less than about 100 microns. The nucleic acid is either RNA, at least 50% (and preferably at least 70% or even 80%) of which is in the form of closed circles, or circular DNA plasmid molecules, at least 50% (and preferably at least 70% or even 80%) of which are supercoiled. In some cases, it is desirable for at least 90% of the microparticles to have a diameter less than about 20 microns, and preferably less than about 11 microns.

Brief Summary Paragraph Right (7):
Another embodiment of the invention features a microparticle less than about 20 microns in diameter, including a polymeric matrix and nucleic acid. The polymeric matrix is made from one or more synthetic polymers having a solubility in water of less than about 1 mg/l. At least 50% (and preferably at least 70% or even 80%) of the nucleic acid molecules are in the form of supercoiled DNA.

Brief Summary Paragraph Right (8):
The polymeric matrix can be biodegradable. Biodegradable is used here to mean that the polymers degrade over time into compounds which are known to be cleared from the host cells by normal metabolic pathways. Generally, a biodegradable polymer will be substantially metabolized within about 1 month after injection into a patient, and certainly within about 2 years. In certain cases, the polymeric matrix can be made of a single synthetic, biodegradable copolymer, e.g., poly-lactic-co-glycolic acid (PLGA). The ratio of lactic acid to glycolic acid in the copolymer can be within the range of about 1:2 to about 4:1 by weight, preferably within the range of about 1:1 to about 2:1 by weight, and most preferably about 65:35 by weight. In some cases, the polymeric matrix also includes a targeting molecule such as a ligand, receptor, or antibody, to increase the specificity of the microparticle for a given cell type or tissue type.

Brief Summary Paragraph Right (9):

For certain applications, the microparticle has a diameter of less than about 11 microns. The microparticle can be suspended in an aqueous solution (e.g., for delivery by injection) or can be in the form of a dry solid (e.g., for storage or for delivery via inhalation or implantation). The nucleic acid can be an expression control sequence operatively linked to a coding sequence. Expression control sequences include, for example, any nucleic acid sequences known to regulate transcription or translation, such as promoters, enhancers, or silencers. In preferred examples, at least 60% or 70% of the DNA is supercoiled. More preferably, at least 80% is supercoiled.

Brief Summary Paragraph Right (10):
In another embodiment, the invention features a microparticle less than about 20 microns in diameter, including a polymeric matrix and a nucleic acid molecule (preferably in closed, circular form), wherein the nucleic acid molecule includes an expression control sequence operatively linked to a coding sequence. The expression product encoded by the coding sequence can be a polypeptide at least 7 amino acids in length, having a sequence essentially identical to the sequence of either a fragment of a naturally-occurring mammalian protein or a fragment of a naturally-occurring protein from an agent which infects or otherwise harms a mammal; or a peptide having a length and sequence which permit it to bind to an MHC class I or II molecule. Examples are set forth in WO 94/04171, herein incorporated by reference.

Brief Summary Paragraph Right (17):
In another embodiment, the invention features a microparticle less than about 20 microns in diameter, including a polymeric matrix and a nucleic acid molecule, wherein the nucleic acid molecule includes an expression control sequence operatively linked to a coding sequence. The expression product encoded by the coding sequence is a

Brief Summary Paragraph Right (18):
In another embodiment, the invention features a process for preparing microparticles. A first solution, including a polymer dissolved in an organic solvent, is mixed (e.g., with sonication) with a second solution, which includes a nucleic acid dissolved or suspended in a polar or hydrophilic solvent. The mixture forms a first emulsion. The first emulsion is then mixed with a third solution which includes an organic compound, to form a second emulsion containing microparticles of polymer matrix and nucleic acid. The mixing steps can be executed, for example, in a homogenizer, vortex mixer, or sonicator. Both mixing steps are carried out in a manner that minimizes shearing of the nucleic acid while producing microparticles on average smaller than 100 microns in diameter.

Brief Summary Paragraph Right (20):
The procedure can include the additional step of washing the microparticles with an aqueous solution to remove organic solvent, thereby producing washed microparticles. The washed microparticles can then be subjected to a temperature below 0.degree. C., to produce frozen microparticles, which are in turn lyophilized to produce lyophilized microparticles.

Brief Summary Paragraph Right (21):
When desired, the procedure can include the additional step of screening the microparticles to remove those larger than 100 microns (or even 20 microns) in diameter.

Brief Summary Paragraph Right (22):
Still another embodiment of the invention features a preparation of microparticles which include a polymeric matrix, a proteinaceous antigenic determinant or other protein (e.g., one which up- or down-regulates immune responses), and a DNA molecule which encodes an antigenic polypeptide that can be different from, or the same as, the aforementioned protein/antigenic determinant. The antigenic determinant contains an epitope which can elicit an antibody response. The antigenic polypeptide expressed from the DNA can induce a T cell response (e.g., a CTL response). The DNA can be plasmid DNA, and can be combined in the same microparticle as the protein/antigenic determinant, or the two can be in distinct microparticles which are then mixed together. In another embodiment, the invention features a method of

administering nucleic acid to an animal by introducing into the animal (e.g., a mammal such as a human, non-human primate, horse, cow, pig, sheep, goat, dog, cat, mouse, rat, guinea pig, hamster, or ferret) any of the microparticles described in the paragraphs above. The microparticles can be provided suspended in a aqueous solution or any other suitable formulation, and can be, for example, injected or implanted (e.g., surgically) into the animal. They can optionally be delivered in conjunction with a protein such as a cytokine, an interferon, or an antigen.

Drawing Description Paragraph Right (2):
FIG. 2 is a plot of size distribution of DNA-containing microparticles as analyzed on a COULTER.TM. counter.

Drawing Description Paragraph Right (4):
FIGS. 4A and 4B are a pair of FACS printouts comparing cell populations in the absence or presence of microparticles.

Detailed Description Paragraph Right (1):
The microparticles of the invention are formulated in one of two ways: (1) to maximize delivery into the patient's phagocytic cells, or (2) to form a deposit in the tissues of the patient, from which the nucleic acid is released gradually over time; upon release from the microparticle, the nucleic acid is taken up by neighboring cells (including APCs) as free DNA. In both cases, maintaining the integrity of the DNA is a priority. For plasmid DNA, this means maximizing the percentage of plasmid molecules that are supercoiled and thus capable of more efficient transfection than non-supercoiled (i.e., nicked or linear) plasmids. Means for protecting the integrity of the nucleic acid include minimizing the shearing forces to which the nucleic acid is necessarily exposed in the process of microparticle formation, and limiting sonication times during preparation. For example, it is necessary to achieve a balance between sonication time and intensity. These techniques are discussed below.

Detailed Description Paragraph Right (2):
Phagocytosis of microparticles by macrophages and other antigen presenting cells (APCs) is an effective means for introducing the nucleic acid into these cells. Phagocytosis by these cells can be increased by maintaining a particle size below about 20 .mu.m, and preferably below about 11 .mu.m. The type of polymer used in the microparticle can also affect the efficiency of uptake by phagocytic cells, as discussed below.

Detailed Description Paragraph Right (3):
The microparticles can be delivered directly into the bloodstream (i.e., by intravenous or intraarterial injection or infusion) if uptake by the phagocytic cells of the reticuloendothelial system (RES) is desired. Alternatively, one can target, via subcutaneous injection, take-up by the phagocytic cells of the draining lymph nodes. The microparticles can also be introduced intradermally (i.e., to the APCs of the skin, such as dendritic cells and Langerhans cells). Another useful route of delivery (particularly for DNAs encoding tolerance-inducing polypeptides) is via the gastrointestinal tract, e.g., orally. Finally, the microparticles can be introduced into the lung (e.g., by inhalation of powdered microparticles or of a nebulized or aerosolized solution containing the microparticles), where the particles are picked up by the alveolar macrophages.

Detailed Description Paragraph Right (4):
Once a phagocytic cell phagocytoses the microparticle, the nucleic acid is released into the interior of the cell. Upon release, it can perform its intended function: for example, expression by normal cellular transcription/translation machinery (for an expression vector), or alteration of cellular processes (for antisense or ribozyme molecules).

Detailed Description Paragraph Right (5):
Because these microparticles are passively targeted to macrophages and other types of phagocytic cells, they represent a means for modulating immune function. Macrophages serve as professional APCs, expressing both MHC class I and class II molecules. Delivery, via microparticles, of an expression vector encoding a foreign antigen which binds to an MHC class I or class II molecule will induce a host T cell

response against the antigen, thereby conferring host immunity.

Detailed Description Paragraph Right (8):
Induction of immune responses can require several factors. It is this multifactorial nature that provides impetus for attempts to manipulate immune related cells on multiple fronts, using the microparticles of the invention. For example, microparticles can be prepared which carry both DNA and polypeptides within each microparticle. Alternatively, a mixture of microparticles can be used, some of which contain DNA and the rest of which contain polypeptide. These dual-function microparticle preparations are discussed below.

Detailed Description Paragraph Right (10):
Alternatively, proteins which promote migration of lymphocytes and macrophages to a particular area could be included in microparticles along with appropriate DNA molecules. Uptake of the DNA is enhanced as a result, because release of the protein would cause an influx of phagocytic cells and T cells as the microparticle degrades. The macrophages would phagocytose the remaining microparticles and act as APC, and the T cells would become effector cells.

Detailed Description Paragraph Right (12):
In general, antibody responses are directed against conformational determinants and thus require the presence of a protein or a protein fragment containing such a determinant. In contrast, T cell epitopes are linear determinants, typically just 7-25 residues in length. Thus, when there is a need to induce both a CTL and an antibody response, the microparticles can include both an antigenic protein and the DNA encoding a CTL epitope.

Detailed Description Paragraph Right (13):
Slow release of the protein from microparticles would lead to B cell recognition and subsequent secretion of antibody. In contrast, phagocytosis of the microparticles would cause APCs (1) to express the DNA of interest, thereby generating a T cell response; and (2) to digest the protein released from the microparticles, thereby generating peptides which are subsequently presented by class II molecules. Presentation by class II molecules promotes both antibody and CTL responses, since T.sub.H cells activated by the class II/peptide complexes would secrete non-specific cytokines.

Detailed Description Paragraph Right (14):
Certain immune responses lead to allergy and autoimmunity, and so can be deleterious to the host. In these instances, there is a need to inactivate tissue-damaging immune cells. Immunosuppression can be achieved with microparticles bearing DNA which encodes epitopes that down regulate T.sub.H cells and CTLs (e.g., "blocking" peptides). In these microparticles, the effect of the immunosuppressive DNA could be amplified by including certain proteins in the carrier microparticles with the DNA. A list of such proteins includes antibodies, receptors, and the interleukins.

Detailed Description Paragraph Right (16):
Inclusion of proteins comprising soluble forms of costimulatory molecules (e.g., CD-40, gp-39, B7-1, and B7-2) is another way to inhibit activation of particular T cell and/or B cells responses. For example, B7-1 is involved in the activation of T.sub.H 1 cells, and B7-2 activates T.sub.H.sup.2 cells. Depending on the response that is required, one or the other of these proteins could be included in the microparticle with the DNA, or could be supplied in separate microparticles mixed with the DNA-containing microparticles Microparticles containing just proteins can be prepared by standard methods; preparation of microparticles containing both protein and DNA is discussed below.

Detailed Description Paragraph Right (17):
A second microparticle formulation of the invention is intended not to be taken up directly by cells, but rather to serve primarily as a slow-release reservoir of nucleic acid that is taken up by cells only upon release from the microparticle through biodegradation. The polymeric particles in this embodiment should therefore be large enough to preclude phagocytosis (i.e., larger than 10 .mu.m and preferably larger than 20 .mu.m). Such particles are produced by the methods described above for making the smaller particles, but with less vigorous mixing of the

aforementioned first or second emulsions. That is to say, a lower homogenization speed, vortex mixing speed, or sonication setting can be used to obtain particles having a diameter around 100 .mu.m rather than 10 .mu.m. The time of mixing also can be altered.

Detailed Description Paragraph Right (18):
The larger microparticles can be formulated as a suspension, a powder, or an implantable solid, to be delivered by intramuscular, subcutaneous, intradermal, intravenous, or intraperitoneal injection; via inhalation (intranasal or intrapulmonary); orally; or by implantation. These particles are useful for delivery of any expression vector or other nucleic acid for which slow release over a relatively long term is desired: e.g., an antisense molecule, a gene replacement therapeutic, a means of delivering cytokine-based, antigen-based, or hormone-based therapeutic, or an immunosuppressive agent. The rate of degradation, and consequently of release, varies with the polymeric formulation. This parameter can be used to control immune function. For example, one would want a relatively slow release of antigen to elicit secretion of IL-4 or IL-10, and a relatively rapid release of antigen to elicit secretion of IL-2 or .gamma.-IFN.

Detailed Description Paragraph Right (24):
During this process, the polymer forms into minute "microparticles," each of which contains some of the nucleic acid-containing solution. If desired, one can isolate a small amount of the nucleic acid at this point in order to assess integrity, e.g., by gel electrophoresis.

Detailed Description Paragraph Right (26):
This process forms a second emulsion which is subsequently added to another organic solution with stirring (e.g., in a homogenizer). In a preferred method, the latter solution is 0.05% w/v PVA. The resultant microparticles are washed several times with water to remove the organic compounds. Particles can be passed through sizing screens to selectively remove those larger than the desired size. If the size of the microparticles is not crucial, one can dispense with the sizing step. After washing, the particles can either be used immediately or be lyophilized for storage.

Detailed Description Paragraph Right (28):
The size distribution of the microparticles prepared by the above method can be determined with a COULTERM.TM. counter. This instrument provides a size distribution profile and statistical analysis of the particles. Alternatively, the average size of the particles can be determined by visualization under a microscope fitted with a sizing slide or eyepiece.

Detailed Description Paragraph Right (29):
If desired, the nucleic acid can be extracted from the microparticles for analysis by the following procedure. Microparticles are dissolved in an organic solvent such as chloroform or methylene chloride in the presence of an aqueous solution. The polymer stays in the organic phase, while the DNA goes to the aqueous phase. The interface between the phases can be made more distinct by centrifugation. Isolation of the aqueous phase allows recovery of the nucleic acid. To test for degradation, the extracted nucleic acid can be analyzed by HPLC or gel electrophoresis.

Detailed Description Paragraph Right (30):
To increase the recovery of nucleic acid, additional organic solvents, such as phenol and chloroform, can be added to the dissolved microparticles, prior to the addition of the aqueous solution. Following addition of the aqueous solution, the nucleic acid enters the aqueous phase, which can easily be partitioned from the organic phase after mixing. For a clean interface between the organic and aqueous phases, the samples should be centrifuged. The nucleic acid is retrieved from the aqueous phase by precipitation with salt and ethanol in accordance with standard methods.

Detailed Description Paragraph Right (31):
Microparticles containing DNA are resuspended in saline, buffered salt solution, or tissue culture medium. For in vitro/ex vivo use, the suspension of microparticles can be added either to cultured adherent mammalian cells or to a cell suspension. Following a 1-24 hour period of incubation, those particles not taken up are removed

by aspiration or centrifugation over fetal calf serum. The cells can be either analyzed immediately or recultured for future analysis.

Detailed Description Paragraph Right (32):
Uptake of microparticles containing nucleic acid into the cells can be detected by PCR, or by assaying for expression of the nucleic acid. For example, one could measure transcription of the nucleic acid with a Northern blot, reverse transcriptase PCR, or RNA mapping. Protein expression can be measured with an appropriate antibody-based assay, or with a functional assay tailored to the function of the polypeptide encoded by the nucleic acid. For example, cells expressing a nucleic acid encoding luciferase can be assayed as follows: after lysis in the appropriate buffer (e.g., cell lysis culture reagent, Promega Corp, Madison Wis.), the lysate is added to a luciferin containing substrate (Promega Corp) and the light output is measured in a luminometer or scintillation counter. Light output is directly proportional to the expression of the luciferase gene.

Detailed Description Paragraph Right (33):
If the nucleic acid encodes a peptide known to interact with a class I or class II MHC molecule, an antibody specific for that MHC molecule/peptide complex can be used to detect the complex on the cell surface of the cell, using a fluorescence activated cell sorter (FACS). Such antibodies can be made using standard techniques (Murphy et al. Nature, Vol. 338, 1989, pp. 765-767). Following incubation with microparticles containing a nucleic acid encoding the peptide, cells are incubated for 10-120 minutes with the specific antibody in tissue culture medium. Excess antibody is removed by washing the cells in the medium. A fluorescently tagged secondary antibody, which binds to the first antibody, is incubated with the cells. These secondary antibodies are often commercially available, or can be prepared using known methods. Excess secondary antibody must be washed off prior to FACS analysis.

Detailed Description Paragraph Right (37):
Microparticles containing nucleic acid can be injected into mammals intramuscularly, intravenously, intraarterially, intradermally, intraperitoneally, or subcutaneously, or they can be introduced into the gastrointestinal tract or the respiratory tract, e.g., by inhalation of a solution or powder containing the microparticles. Expression of the nucleic acid is monitored by an appropriate method. For example, expression of a nucleic acid encoding an immunogenic protein of interest is assayed by looking for an antibody or T cell response to the protein.

Detailed Description Paragraph Right (38):
Antibody responses can be measured by testing serum in an ELISA assay. In this assay, the protein of interest is coated onto a 96 well plate and serial dilutions of serum from the test subject are pipetted into each well. A secondary, enzyme-linked antibody, such as anti-human, horseradish peroxidase-linked antibody, is then added to the wells. If antibodies to the protein of interest are present in the test subject's serum, they will bind to the protein fixed on the plate, and will in turn be bound by the secondary antibody. A substrate for the enzyme is added to the mixture and a colorimetric change is quantitated in an ELISA plate reader. A positive serum response indicates that the immunogenic protein encoded by the microparticle's DNA was expressed in the test subject, and stimulated an antibody response. Alternatively, an ELISA spot assay can be employed in order to look at B cells specifically.

Detailed Description Paragraph Right (39):
T cell proliferation in response to a protein following intracellular delivery of microparticles containing nucleic acid encoding the protein is measured by assaying the T cells present in the spleen, lymph nodes, or peripheral blood lymphocytes of a test animal. The T cells obtained from such a source are incubated with syngeneic APCs in the presence of the protein or peptide of interest. Proliferation of T cells is monitored by uptake of .sup.3 H-thymidine, according to standard methods. The amount of radioactivity incorporated into the cells is directly related to the intensity of the proliferative response induced in the test subject by expression of the microparticle-delivered nucleic acid. A positive response indicates that the microparticle containing DNA encoding the protein or peptide was taken up and expressed by APCs in vivo.

Detailed Description Paragraph Right (40):
The generation of cytotoxic T cells can be demonstrated in a standard .sup.51 Cr release assay. In these assays, spleen cells or peripheral blood lymphocytes obtained from the test subject are cultured in the presence of syngeneic APCs and either the protein of interest or an epitope derived from this protein. After a period of 4-6 days, the effector cytotoxic T cells are mixed with .sup.51 Cr-labeled target cells expressing an epitope derived from the protein of interest. If the test subject raised a cytotoxic T cell response to the protein or peptide encoded by the nucleic acid contained within the microparticle, the cytotoxic T cells will lyse the targets. Lysed targets will release the radioactive .sup.51 Cr into the medium. Aliquots of the medium are assayed for radioactivity in a scintillation counter. Assays, such as ELISA, can also be used to measure cytokine profiles.

Detailed Description Paragraph Right (42):
Plasmid DNA was prepared by standard methods using MEGA-PREP.TM. Kit (Qiagen) according to the manufacturer's instructions. An endotoxin-free buffer kit (Qiagen) was used for all DNA manipulations. The DNA was resuspended in distilled, deionized, sterile water to give a final concentration of 3 .mu.g/.mu.l. FIG. 1 shows plasmid maps of DNA expression vectors encoding a) luciferase, b) a vesicular stomatitis virus (VSV) peptide epitope termed VSV-Npep, and c) a human papilloma virus (HPV) peptide epitope termed A2.1/4.

Detailed Description Paragraph Right (44):
The microparticle solution was poured into a 250 ml centrifuge tube and spun at 2000 rpm for 10 minutes. The contents of the tubes were decanted and the sedimented particles were resuspended in 100 ml deionized water. After repeating the centrifugation and decanting steps, the particles were frozen in liquid nitrogen and finally lyophilized until dry.

Detailed Description Paragraph Right (45):
5 mg of the lyophilized microparticles were resuspended in 200 .mu.l water. The resulting suspension was diluted to about 1:10,000 for analysis with a COULTERM counter. FIG. 2 is a print-out from the COULTER.TM. counter which indicates that approximately 85% of the microparticles were between 1.1 and 10 .mu.m in diameter.

Detailed Description Paragraph Right (46):
2-5 .mu.g of the microparticles were wet with 10 .mu.l water in an EPPENDORF.TM. tube. 500 .mu.l chloroform was added with thorough mixing to dissolve the polymeric matrix. 500 .mu.l water was added, again with mixing. The resulting emulsion was centrifuged at 14,000 rpm for 5 minutes. The aqueous layer was transferred to a clean EPPENDORF.TM. tube, along with 2 volume equivalents of ethanol and 0.1 volume equivalents of 3M aqueous sodium acetate. The mixture was centrifuged at 14,000 rpm for 10 minutes. After aspiration of the supernatant, the pelleted DNA was resuspended in 50 .mu.l water. 5 .mu.g DNA was electrophoresed on a 0.8% agarose gel next to a standard containing the input DNA. The DNA on the gel was visualized on a UV light box. Comparison with the standard gives an indication of the integrity of the microparticles' DNA. The microparticle formation procedure was deemed successful if the incorporated DNA retained a high percentage of supercoiled DNA relative to the input DNA.

Detailed Description Paragraph Right (47):
As indicated in FIGS. 3A and 3B, homogenization speed and duration are inversely related to DNA integrity. FIG. 3A depicts the DNA isolated from microparticles prepared by homogenization at 7000 rpm for 1 minute (lane 1), and supercoiled input DNA (lane 2). FIG. 3B shows DNA isolated from microparticles prepared by homogenization at 7000 rpm for 5 seconds (lane 1), DNA isolated from microparticles prepared by homogenization at 5000 rpm for 1 minute (lane 2), and supercoiled input DNA (lane 3).

Detailed Description Paragraph Right (48):
Into each of two wells of a six-well tissue culture dish, about 10.sup.6 macrophages were plated in 3 ml RPMI medium containing 10% fetal calf serum. 5 mg of the microparticles containing DNA encoding luciferase were resuspended in 200 .mu.l saline solution, and 50 .mu.l of the resulting suspension was added to one of the

wells containing macrophages. The plate was incubated at 37.degree. C. for 1-6 hours. Side vs. forward scatter (i.e., intracellular complexity vs. size) of the cells was analyzed by FACS using a Becton Dickinson FACS instrument.

Detailed Description Paragraph Right (50):
Into two wells of a 24-well tissue culture dish, about 2.5.times.10.sup.5 macrophages were plated in 1 ml RPMI medium containing 10% fetal calf serum. The plate was incubated at 37.degree. C. for 6 hours. 1 mg of the lyophilized microparticles containing DNA encoding luciferase was resuspended in 400 .mu.l saline solution. 6 .mu.l of the resulting suspension was added to one of the wells containing macrophages, and 25 .mu.l of suspension was added to the other. The plate was incubated at 37.degree. C. for 4 hours. The medium, including the microparticles, was removed and fresh medium added to the cells. The plate was again incubated at 37.degree. C. for 1-5 days. The cells were harvested into a tube and spun at 1,500 RPM for 5 minutes. The pelleted cells were resuspended in 100 .mu.l of 1.times. Cell Lysis Buffer (Promega) in an EPPENDORF.TM. tube. The mixture was centrifuged at 14,000 RPM for 5 minutes in order to precipitate out any cell debris. The cell lysate was assayed by adding 5 .mu.l of the supernatant to 100 .mu.l of luciferase substrate (Promega) and measuring the light output on a TOPCOUNT.TM. combination luminometer/scintillation counter (Packard Instruments).

Detailed Description Paragraph Right (51):
The data for this experiment are provided in Table 5. They indicate that cells phagocytosing microparticles that contain, for example, luciferase DNA, do in fact express the DNA. Thus, DNA integrity and functionality are confirmed. The data also indicate that the uptake of the microparticles by phagocytosis does not prevent the DNA from reaching the nucleus.

Detailed Description Paragraph Right (52):
45 mg of luciferase cDNA in microparticles was resuspended in 250 .mu.l saline solution. 40 .mu.l of the resulting suspension was injected into each tibialis anterior muscle of a mouse. Seven days later, each tibialis anterior was dissected and placed in an EPPENDORF.TM. tube on dry ice. Using a mortar and pestle cooled with dry ice, each tibialis anterior muscle was ground into a powder, then return to the EPPENDORF.TM. tube. 500 .mu.l 1.times. cell lysis buffer (Promega) was added. The tube was shaken upside-down on a vortex mixer at 40.degree. C. for 15 minutes. The tube and its contents were frozen in liquid nitrogen, then thawed to 37.degree. C. The freeze/thaw cycle was repeated two more times. The tube was centrifuged 14,000 RPM for 10 minutes. The supernatant was transferred to a new tube and centrifuged again for 5 minutes. To assay for expression, 20 .mu.l of the supernatant was added to 100 .mu.l of luciferase substrate (Promega) and the light output was measured on a TOPCOUNT.TM. combination luminometer/scintillation counter (Packard Instruments).

Detailed Description Paragraph Right (53):
The data for this experiment are provided in Table 6. They indicate that muscle cells can express DNA released from microparticles. Since these cells are not known to phagocytose, this is an example of depot effect.

Detailed Description Paragraph Right (54):
90 mg of microparticles containing DNA encoding VSV-Npep was resuspended in 900 .mu.l of saline solution. 60 mg of microparticles containing control vector DNA was resuspended in 600 .mu.l of saline solution. 300 .mu.g VSV-Npep plasmid DNA was resuspended in 300 .mu.l of saline solution. 300 .mu.g control vector DNA was resuspended in 300 .mu.l of saline solution. 150 .mu.g of the VSV-N peptide was resuspended in incomplete Freund's adjuvant (IFA).

Detailed Description Paragraph Right (56):
After two weeks, groups 5, 6, and 8, which received either synthetic peptide or DNA without microparticles, were injected again. Groups 1-4 and 7, which initially received microparticles, were not reinjected.

Detailed Description Paragraph Right (61):
In the experiment associated with FIG. 5, effector cells from mice (Group 1) immunized intraperitoneally with microparticles containing DNA that encodes a

peptide from the VSV-N protein were tested for cytolytic activity against various target cells. The VSV peptide binds to the mouse H-2K.sup.b class I receptor. Syngeneic targets express the H-2K.sup.b receptor while the allogeneic targets used in this experiment express the H-2K.sup.d receptor.

Detailed Description Paragraph Right (64):
Together, the data demonstrate that CTL activity can be elicited by immunization with microparticles containing DNA that encodes a VSV peptide, and the lysis is MHC restricted and peptide specific. In other words, only the right peptide with the right MHC receptor is recognized by the T cell receptor of the CTL generated by immunization in accordance with the invention. This demonstrated that the microparticles serve the desired function.

Detailed Description Paragraph Right (65):
Next, the CTL response generated by immunizing mice subcutaneously with synthetic peptide (Group 8) was compared with the CTL response generated by immunizing mice intraperitoneally with microparticles containing DNA that encodes the VSV peptide (Groups 1 and 2). In FIG. 6 is shown the lysis obtained at a E:T ratio of 100:1 for CTL generated by immunizing the mice with either microparticles including DNA that encodes the VSV-N peptide (MS-VSV; Group 1), microparticles including control vector DNA that does not encode a VSV peptide (MS-vector; Group 2), or synthetic VSV-N peptide (peptide; Group 8). The targets were syngeneic (EL4) cells labelled with VSV peptide.

Detailed Description Paragraph Right (66):
Mice immunized with the VSV-Npep DNA in microparticles (MS-VSV) generated a stronger CTL response (33% specific lysis) than mice immunized with control microparticles containing empty vector DNA (MS-vector) (10% specific lysis). Mice immunized with VSV-N peptide (peptide) generate a weaker CTL response than those immunized with microparticles containing VSV-Npep DNA (MS-VSV). Therefore, the microparticles served the desired function.

Detailed Description Paragraph Right (67):
CTL responses in mice immunized intraperitoneally with VSV-Npep DNA contained in microparticles (MS-VSV) were compared with the CTL responses of mice immunized intramuscularly with "naked" VSV DNA (VSV). CTL responses in mice immunized with the microparticles containing DNA (MS-VSV; Group 1) were stronger than those in mice immunized with naked DNA (VSV; Group 5) at an E:T ratio of 3:1 (FIG. 7). The targets were syngeneic (EL4) cells labelled with VSV peptide. The mice which received naked DNA were immunized twice, while the mice immunized with microparticles were only given one treatment. The data in FIG. 7 therefore show that one injection of DNA in microparticles was more effective than two injections of a greater amount of naked DNA.

Detailed Description Paragraph Right (68):
FIG. 8 shows the results of an experiment equivalent to that related in FIG. 5, with the exception that the injections were subcutaneous (Group 7 mice) instead of intraperitoneal. This experiment demonstrated that subcutaneous injections of microparticles containing VSV-Npep DNA are also effective for producing CTL responses.

Detailed Description Paragraph Right (69):
The experiment illustrated in FIG. 9 is also similar to that of FIG. 5, except that DNA encoding a different peptide was used in order to demonstrate that the results obtained were not unique to VSV-Npep DNA. HLA-A2 transgenic mice were immunized with microparticles containing DNA that encodes a peptide from human papillomavirus (HPV) E6 peptide. The HPV E6 peptide termed A2.1/4 binds to the human MHC receptor HLA-A2. The experiment assessed the ability of CTL effectors to lyse syngeneic targets (i.e., targets having the correct HLA receptor) that were either labeled with the correct HPV peptide (A2.1/4) or else unlabeled (no peptide). The E:T ratios are listed along the X-axis.

Detailed Description Paragraph Right (70):
According to the procedure of example 1, microparticles are prepared containing DNA encoding a peptide having an amino acid sequence about 50% identical to PLP residues

170-191 (SEQ ID NO: 2). A multiple sclerosis patient whose T cells secrete excess T.sub.H 1 cytokines (i.e., IL-2 and .gamma.-IFN) in response to autoantigens is injected intravenously with 100 .mu.l to 10 ml of the microparticles. Expression of the PLP-like peptide by APCs results in the switching of the cytokine profile of the T cells, such that they instead produce T.sub.H 2 cytokines (i.e., IL-4 and IL-10) in response to autoantigens.

Detailed Description Paragraph Right (71):
According to the procedure of example 1, microparticles are prepared containing DNA encoding a peptide having an amino acid sequence corresponding to MBP residues 33-52 (SEQ ID NO: 34). A mammal is injected subcutaneously with 50-500 .mu.l of the microparticles. Expression of the MBP peptide by APCs results in the tolerization of T cells that recognize the autoantigen.

Detailed Description Paragraph Right (72):
A DNA molecule, including an expression control sequence operatively linked to a sequence encoding both a trafficking sequence and a peptide essentially identical to myelin basic protein (MBP) residues 80-102 (SEQ ID NO: 1), is associated with a polymer to form microparticles, according to the procedure of example 1. Particles smaller than 100 .mu.m are removed. The polymeric constituent of the microparticle is poly-lactic-co-glycolic acid, where the ratio of lactic acid to glycolic acid is 65:35 by weight. The resulting microparticles are surgically implanted subcutaneously in a patient.

Detailed Description Paragraph Right (74):
Up to 400 mg of PLGA (i.e., at least ten times the mass of protein) is dissolved in about 7 ml methylene chloride. The DNA/protein solution is poured into the PLGA solution and homogenized or sonicated to form a first emulsion. The first emulsion is poured into about 50-100 ml of an aqueous solution of surfactant (e.g., 0.05% to 2% PVA by weight). The mixture is homogenized at about 3000-8000 RPM to form a second emulsion. The microparticles are then isolated according to the procedure of example 1.

Detailed Description Paragraph Right (75):
Microparticles including both an antigenic protein having the conformational determinants necessary for induction of B cell response against hepatitis B virus (HBV) and DNA encoding the CTL epitope for HBV, are prepared according to the procedure of example 6. A patient infected or at risk of infection with HBV is immunized with the microparticles.

Detailed Description Paragraph Right (76):
Slow release of the protein from non-phagocytosed microparticles leads to B cell recognition of the conformational determinants and subsequent secretion of antibody. Slow release of the DNA or phagocytosis of other microparticles causes APCs (1) to express the DNA of interest, thereby generating a T cell response; and (2) to digest the protein released from the microparticles, thereby generating peptides which are subsequently presented by class I or II molecules. Presentation by class I molecules promotes CTL response; presentation by class II molecules promotes both antibody and T cell responses, since T.sub.H cells activated by the class II/peptide complexes secrete non-specific cytokines.

Detailed Description Paragraph Left (4):
Microparticles for Implantation

Detailed Description Paragraph Left (6):
Characterization of Microparticles

Detailed Description Paragraph Left (7):
Intracellular Delivery of Microparticles

Detailed Description Paragraph Left (8):
In Vivo Delivery of Microparticles

Detailed Description Paragraph Left (12):
Analysis of Microparticle Size Profile

Detailed Description Paragraph Left (15):
In Vitro Phagocytosis of DNA-Containing Microparticles

Detailed Description Paragraph Left (19):
Generation of Cytotoxic T Cells Following Injection of Microparticles Containing DNA

Detailed Description Paragraph Left (20):
Treatment with Microparticles Containing DNA

Detailed Description Paragraph Left (21):
Tolerizing with Microparticles Containing DNA

Detailed Description Paragraph Left (22):
Implantation of Microparticles

Detailed Description Paragraph Left (23):
Preparation of Microparticles Containing Both DNA and Protein

Detailed Description Paragraph Left (24):
Treatment with Microparticles Containing Both DNA and Protein

Detailed Description Paragraph Type 0 (1):
1. Intraperitoneal: A first group of 3 mice was injected intraperitoneally with 100 .mu.l of microparticles containing VSV-Npep DNA (Group 1). A second group of 3 mice was injected with 100 .mu.l of microparticles containing control vector DNA (Group 2).

Detailed Description Paragraph Type 0 (2):
2. Intramuscular: (into each tibialis anterior muscle): A third group of 3 mice was injected intramuscularly with 100 .mu.l of microparticles containing VSV-Npep DNA (Group 3). A fourth group of 3 mice was injected with 100 .mu.l microparticles containing control vector DNA (Group 4). A fifth group of 3 mice was injected with 50 .mu.g/leg VSV-Npep plasmid DNA (i.e., in the absence of microparticles) (Group 5). A sixth group of 3 mice was injected with 50 .mu.g/leg control vector plasmid DNA (Group 6). 3. Subcutaneous: A seventh group of 3 mice was injected subcutaneously with 100 .mu.l of microparticles containing VSV-Npep DNA (Group 7). An eighth group of 3 mice was injected with 50 .mu.g VSV-N peptide/IFA (Group 8).

Detailed Description Paragraph Table (1):
TABLE 5 Phagocytosis of encapsulated DNA leads to expression of a luciferase reporter gene construct. MICROPARTICLES CONTAINING: Luciferase DNA Control DNA 25 .mu.l 6 .mu.l 25 .mu.l 6 .mu.l

Day 1	1257	168	103	245	Day 2	2632	492	107	133
Day 3	3400	507	80	93	Day 5	763	310	90	90

Data given in counts per 0.01 minute

Other Reference Publication (5):
Jones et al., "Immune Responses Following Oral and Parenteral Administration of Plasmid DNA Encapsulated in Poly(lactide-coglycolide) Microparticles," Int'l Meeting on Nucleic Acid Vaccines, Bethesda, MD, 1996.

CLAIMS:

1. A preparation of microparticles, each of which comprises a polymeric matrix and nucleic acid, the polymeric matrix consisting essentially of one or more synthetic polymers having a solubility in water of less than about 1 mg/l, wherein

at least 90% of the microparticles have a diameter less than about 100 microns, and

the nucleic acid is an expression vector selected from the group consisting of RNA molecules, at least 50% of which are closed circles; and circular plasmid DNA molecules, at least 50% of which are supercoiled.

2. A microparticle less than about 20 microns in diameter, comprising:

a polymeric matrix consisting essentially of one or more synthetic polymers having a solubility in water of less than about 1 mg/l; and

nucleic acid molecules, at least 50% of which are supercoiled DNA.

3. The microparticle of claim 2, wherein the polymeric matrix is biodegradable.

4. The microparticle of claim 2, wherein the polymeric matrix consists essentially of one synthetic, biodegradable copolymer.

5. The microparticle of claim 4, wherein the copolymer is poly-lactic-co-glycolic acid (PLGA).

6. The microparticle of claim 2, wherein the microparticle has a diameter of less than about 11 microns.

7. The microparticle of claim 2, wherein the nucleic acid molecule comprises an expression control sequence operatively linked to a coding sequence.

8. A microparticle less than about 20 microns in diameter, comprising:

a polymeric matrix; and

a nucleic acid molecule comprising an expression control sequence operatively linked to a coding sequence encoding an expression product comprising a polypeptide at least 7 amino acids in length, said polypeptide having the sequence of (a) a fragment of a naturally-occurring mammalian protein or (b) a fragment of a naturally-occurring protein from an infectious agent which infects a mammal, wherein the expression product includes (i) part but not all of the naturally-occurring mammalian protein or (ii) part but not all of the naturally-occurring protein from an infectious agent.

9. The microparticle of claim 8, wherein the polyeptide is immunogenic.

10. The microparticle of claim 8, wherein the expression product (1) comprises an amino acid sequence of a naturally occurring peptide recognized by a T cell; (2) is recognized by the T cell; and (3) alters the cytokine profile of the T cell.

11. A method of administering nucleic acid to an animal, comprising

providing the microparticle of claim 8; and

introducing the microparticle into the animal.

12. A microparticle less than about 20 microns in diameter, comprising:

a polymeric matrix; and

a nucleic acid molecule comprising an expression control sequence operatively linked to a coding sequence, wherein the coding sequence encodes a protein which, when expressed in a macrophage, downregulates an immune response in an animal.

13. A process for preparing microparticles, comprising:

(1) providing a first solution comprising a polymer dissolved in an organic solvent;

(2) providing a second solution comprising a nucleic acid dissolved or suspended in a polar or hydrophilic solvent;

(3) mixing the first and second solutions to form a first emulsion; and

(4) mixing the first emulsion with a third solution comprising an organic compound,

to form a second emulsion comprising microparticles of polymeric matrix and nucleic acid; wherein both mixing steps are carried out in a manner that minimizes shearing of the nucleic acid while producing microparticles having a number average smaller than 100 microns in diameter.

14. A method of administering nucleic acid to an animal, comprising

providing the microparticle of claim 2; and

introducing the microparticle into the animal.

15. A preparation of microparticles, each of which comprises:

a polymeric matrix;

a proteinaceous antigenic determinant; and

DNA which encodes an antigenic polypeptide.

21. A microparticle less than about 20 microns in diameter, comprising:

a polymeric matrix; and

a nucleic acid molecule comprising an expression control sequence operatively linked to a coding sequence encoding an expression product comprising a trafficking sequence linked to a polypeptide, said polypeptide being at least 7 amino acids in length and having the sequence of (a) a fragment of a naturally-occurring mammalian protein or (b) a fragment of a naturally-occurring protein from an infectious agent which infects a mammal, wherein the expression product includes (i) part but not all of the naturally-occurring mammalian protein or (ii) part but not all of the naturally-occurring protein from an infectious agent.

22. The microparticle of claim 21, wherein the polypeptide is immunogenic.

23. The microparticle of claim 21, wherein the polypeptide (1) is recognized by a T cell; and (2) alters the cytokine profile of the T cell.

24. A microparticle less than about 20 microns in diameter, comprising:

a polymeric matrix; and

a nucleic acid molecule comprising an expression control sequence operatively linked to a coding sequence encoding an expression product having a length and sequence which permit it to bind to an MHC class I or II molecule.

25. The microparticle of claim 24, wherein the expression product is immunogenic.

26. The microparticle of claim 24, wherein the expression product (1) is recognized by a T cell; and (2) alters the cytokine profile of the T cell.

27. A microparticle less than about 20 microns in diameter, comprising:

a polymeric matrix; and

a nucleic acid molecule comprising an expression control sequence operatively linked to a coding sequence encoding an expression product consisting of a trafficking sequence linked to a peptide having a length and sequence which permit it to bind to an MHC class I or II molecule.

28. The microparticle of claim 27, wherein the peptide is immunogenic.

29. The microparticle of claim 27, wherein the peptide (1) is recognized by a T cell, and (2) alters the cytokine profile of the T cell.

30. A method of administering nucleic acid to an animal, comprising

providing the microparticle of claim 21; and

introducing the microparticle into the animal.

31. A method of administering nucleic acid to an animal, comprising

providing the microparticle of claim 24; and

introducing the microparticle into the animal.

32. A method of administering nucleic acid to an animal, comprising

providing the microparticle of claim 27; and

introducing the microparticle into the animal.